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Acute Oral Toxicity, Anti-Inflammatory and Anti-pyretic Activities of Methanol Extract of Moringa oleifera LAM in Albino Rats

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ABSTRACT:

Medicinal plants are the part and parcel of human society to combat against different diseases from the dawn of human civilization. According to World Health Organization, approximately 80% population of the developing countries are facing difficulties to afford synthetic drugs and are relying on traditional medicines mainly of plant origin in order to maintain their primary health care needs. *Moringa oleifera* LAM (Moringaceae) has been used as traditional medicines in many tropical and subtropical countries. It's an exceptionally nutritious vegetable with a variety of potential uses in treating rheumatism, venomous bites, and microbial infections. The anti-inflammatory and antipyretic effect of the methanol extract of *Moringa oleifera* (MEMO) was investigated. The anti-inflammatory effect was studied using Plethysmometer, while the antipyretic effect was estimated using Brewer's yeast-induced hyperpyrexia in rats. The results of the present study revealed that oral administration of MEMO exhibited antipyretic and anti-inflammatory effect in the models studied.

Keywords: *Moringa oleifera*, antipyretic, hyperpyrexia, Brewer's yeast, methanol.

1. INTRODUCTION

Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely prescribed because of their effectiveness in the treatment of pain, fever and inflammation. However, their long-term therapeutic use is often associated with adverse effects such as gastrointestinal ulcers, renal failure [1] and cardiovascular disorders [2]. Several antipyretic drugs have been shown to be toxic, such as paracetamol which causes liver damage and the fall of glutathione in the liver [3,4]. The use of natural products and more particularly medicinal plants is becoming an important alternative route to explore in order to discover effective drugs well tolerated [5]. *Moringa oleifera*, often referred to simply as *Moring*a or winged ben, belongs to the monotypic Moringaceae family. It is widespread in subtropical countries, particularly India, is drought-resistant and grows rapidly. It has been used for decades by NGOs for its nutritional virtues. Pharmacologically speaking, *Moringa* leaves have anti-tumor, anti-inflammatory, anti-bacterial, neuroprotective qualities designed to improve brain function [6–8]. It is referred as the "miracle tree, tree of life, and God's Gift to man [9]. These effects are probably due to its richness in phenolic acids and flavonoids [10, 11], which explain why it is integrated *as* herbal medicines in the Mediterranean region. Moreover, this plant prevents gastric ulcers, skin diseases, and bronchitis [12]. Let's not forget that Moringa's high vitamin B content makes it an ally for digestive health. Optimization of gastric acid prevents bloating, constipation and gas. In addition, Moringa's antibiotic and antibacterial properties help treat and prevent infections, this study was conducted for the first time on an endemic Algerian species named *Moringa oleifera* in order to determine its anti-inflammatory and antipyretic capacity. The aim of the present study is to study the acute toxicity and evaluate the anti-inflammatory and antipyretic properties of the methanol extract of the leaves of the plant *Moringa oleifera* Lam (MEMO).

2. MATERIALS AND METHODS

Plant material

The mature leaves of *M. oleifera* were collected from El-Oued (Oued Souf) in the south of Algeria and identified by professor Farid Bekdouche (Faculty of Natural and Life Sciences, University of Batna 2, Algeria).

Animals

Experiments were performed using Wistar rats of both sexes, weighing (140 – 190 g). The animals were obtained from the Pasteur institute, Algiers, Algeria. The animals were kept in polypropylene cages under standardized conditions for 8 days before experiments. The animals were fed with standard diet and water *ad libitum*.

Extraction

M. oleifera leaves were shade-dried and ground into powder; 500 g of powder were successively macerated with hexane, chloroform, ethyl acetate and methanol (solvent of increasing polarity). The methanolic extract was used for the investigation of the acute toxicity study, anti-inflammatory and antipyretic effects.

Acute toxicity study of the extract

Acute oral toxicity study was performed as per the OECD-423 guidelines [13]. Ten Wistar female rats were used for this study; they were divided into two groups of five animals each. The animals were fasted overnight and allowed free access to water, after *M. oleifera* methanol extract was administered orally in a single dose of 2000 mg/kg body weight. Animals were observed individually after dosing at least once every 30 min, periodically during the first 4 h, with special attention given during the first 24 h.

General symptoms of toxicity and mortality in each group were observed within 24 h. Animals that survived after 24 h were observed daily for any signs of delayed toxicity for two weeks. At the end of 14 days observation period, the animals were anaesthetized, and blood samples were collected through cardiac puncture with and without anticoagulant (EDTA) for hematological and biochemical analysis, respectively.

Hematological analysis

Hematological parameters: white blood cell (WBC), hemoglobin (HGB), red blood cell (RBC), hematocrit (HCT), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), lymphocyte %, lymphocytes number (lymph no), Red cell distribution width (RDW), mean volume platelets of (VPM) and platelets (PLT)were determined using a hematology analyzer **(ADVIA® 2120i System).**

Biochemical analysis

For biochemical parameters blood without additive was centrifuged at 3000 × g at 4°C for 10 min, serum was separated and alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, urea, creatinine, glucose, HDL-Cholesterol, LDL-cholesterol, cholesterol and triglycerides were estimated using automated Analyzer **(COBAS INTEGRA® 400 plus).**

Egg albumin induced paw edema in rats

The anti-inflammatory activity of the tested extract was evaluated in male Wistar rats by the egg albumin induced paw edema method [14]. The animals were divided in five groups (n = 5). All groups were kept fasting and allowed free access to water.

Group I was treated orally with normal saline (10 mL/kg), **group II** with diclofenac (30 mg/kg), the rest of groups (**III**, **IV** and **V**) were treated with *M. oleifera* methanol extract at the dose level of 100, 250 and 500 mg/kg p. o. After 1 h, inflammation induced by sub plantar injection of 100µL of undiluted fresh egg albumin into the right hind paw of all rats. The paw thickness of each rat of all groups was measured in mm using digital vernier calipers at 0, 1, 2, 3, and 5 h.

The percentage inhibition of edema by the tested extract and standard drug was calculated in comparison with vehicle control using this following formula:

$$\%\ \text{Inhibition of oedema} = \frac{(\text{tCn} - \text{tC0}) - (\text{tTn} - \text{tT0})}{(\text{tCn} - \text{tC0})} \times\ 100$$

Where **tCn** = paw thickness at particular time point of control animal; **tC0** = paw thickness before induction; **tTn** = paw thickness at particular time point of treated animal; and **tT0** = paw thickness before induction.

Brewer's yeast induced pyrexia

Antipyretic activity was measured by Brewer's induced pyrexia in male Wistar rats [15]. Rats were fasted overnight with free access to water before the experiments. Pyrexia was induced in animals by subcutaneous injection of 20 % brewer's yeast (10 mL/kg) suspended in saline solution into back side of below the nape of the neck. After 17 h of yeast injection, the rectal temperature of each rat was measured using a digital thermometer. Only rats that showed rectal temperature of 38°C and above were selected for the experiments. Rats were divided into five groups. The 1st group was kept as a control (received the normal saline) while the 2nd one was given paracetamol in a dose of 150 mg/kg (standard). The 3rd - 5th groups received orally 100, 250 and 500 mg/kg of the MEMO, and the rectal temperature was measured periodically at 1, 2, 3 and 5 h after drug administration.

Statistical analysis

Data were expressed as the mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Differences between two means were detected using the Student's t-test (acute toxicity test). Data were considered different at significance level of $p < 0.05$.

3. RESULTS

Acute toxicity

The study of acute toxicity in rats shows that the oral administration of a single dose (2000 mg/kg) of MEMO to rats did not bring any signs of toxicity or mortality in treated animals during the 14 days observation period. In addition, there was no significant difference in biochemical and hematological parameters between control and treatment group. This indicates that MEMO was nontoxic in rats up to the dose of 2000 mg/kg of body weight **(Table 1 and Table 2).**

Table 1: Biochemical parameters of control and treated rats during the acute toxicity study.

Biochemical parameters	Control	MEMO 2000 mg/kg BW
Renal profile		
Urea (mg/L)	0.30 ± 0.01	0.32 ± 0.01
Creatinine (mg/L)	4 ± 0.01	3 ± 0.0
Liver profile		
AST(U/L)	131 ± 5.54	124.6 ± 3.36
ALT(U/L)	86.2 ± 7.13	78.9 ± 9.66
Total bilirubin(mg/dL)	0.76 ± 0.04	0.69 ± 0.07
Lipid profile		
Cholesterol (mmol/L)	0.57 ± 0.04	0.62 ± 0.05
Triglycerides (mmol/L)	0.83 ± 0.10	0.95 ± 0.15
HDL-Cholesterol (mmol/L)	0.50 ± 0.03	0.55 ± 0.06
LDL-Cholesterol (mmol/L)	0.06 ± 0.01	0.06 ± 0.01
Glucose (g/L)	0.67 ± 0.03	0.68 ± 0.05

Values are mean±SEM (n=5). No significant compared to control.

Table 2: Hematological parameters of control and treated rats during the acute toxicity study.

Hematology parameters measured	Control	MEMO 2000 mg/kg BW
RBC(106xµL)	7.35±0.14	7.19±0.06
MCV(fL)	55.68±0.85	56.86±0.67
RDW(fL)	13.18±0.26	13.24±0.25
HCT (%)	40.92±0.48	40.71±0.42
PLT (10^3/µL)	614.8±61.76	638.3±50.75
MPV (fL)	6.18±0.09	6.29±0.13
WBC (10^3/µL)	6.10±0.69	6.81±1.10
HGB(g/dL)	14.5±0.17	14.26±0.11

Values are mean±SEM(n=5). No significant compared to control.

MCH(pg)	19.74±0.25	19.98±0.22
MCHC(g/dL)	35.46±0.17	35.33±0.13
Lymphocyte no	4.24±0.51	4.69±0.93
Lymphocyte %	69.08±2.43	68.67±2.73

Effect of the MEMO on egg albumin induced paw edema in rats

Pre-treatment with MEMO exhibited significant anti-inflammatory effect during various assessment times (1–5h) in a dose dependent manner. Remarkably, the highest percentage inhibition of edema was obtained with 500 mg/kg dose (58.79%) at the 5th hour of drug administration, compared with the other dose levels of the extract and the standard drug (Dichlofenac ; $p<0.05$). The percentage rate of effect of MEMO in fresh egg albumin induced rat paw edema test and the average edema for the various groups was demonstrated in **Table 3.**

Table 3: Anti-oedematous effect of MEMO on egg-albumin induced paw edema in rats.

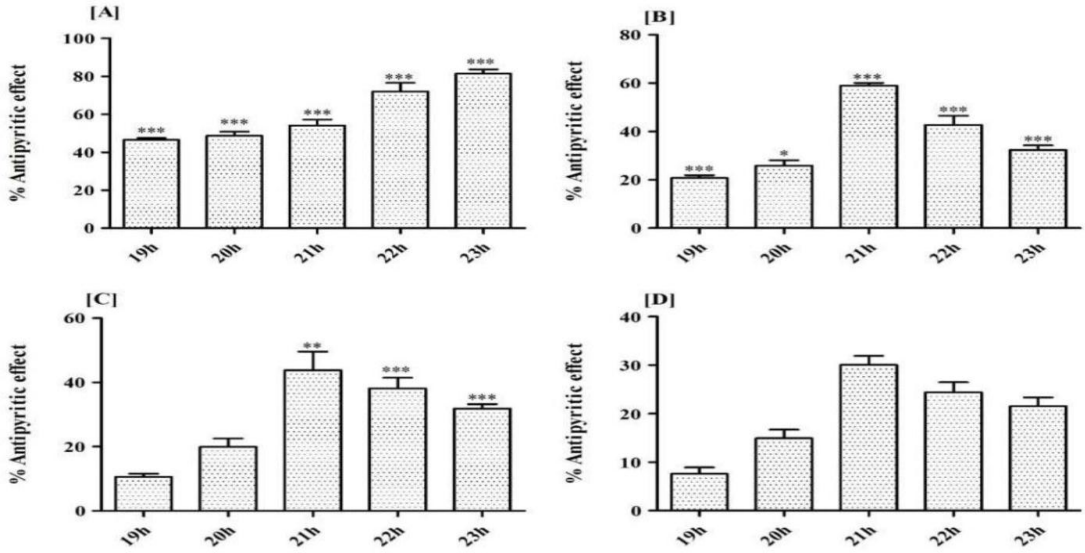
Treatment Dose (mg/kg)	**Δ paw edema (mm) and % inhibition**				
	1h	**2h**	**3h**	**4h**	**5h**
Normal saline10(mL/kg)	5.43±0.11	4.92±0.21	4.64±0.15	4.09±0.17	3.80±0.13
MEMO 100	5.22±0.11 (4.51%)	4.39±0.21 (9.43%)	3.90±0.15** (17.59%)	2.87±0.112*** (32.01%)	2.42±0.11*** (37.77%)
MEMO 250	4.98±0.08 (7.68%)	4.32±0.18 (12.88%)	3.71±0.15*** (20.95%)	2.65±0.10*** (37.05%)	1.88±0.07*** (51.79%)
MEMO 500	4.88±0.11*** (12.79%)	3.98±0.18*** (21.22%)	3.31±0.10*** (32.03%)	2.37±0.08*** (45.23%)	1.71±0.05*** (58.79%)
Dichlofenac 30	4.85±0.07*** (10.65%)	3.87±0.16*** (21.43%)	2.71±0.08*** (41.59%)	1.94±0.07*** (52.31%)	1.24±0.03*** (67.28%)

Values are mean±SEM(n=5). *P<0.05, **P<0.01, ***P<0.001compared to control.

Antipyretic effect of MEMO in Brewer's yeast induced pyrexia test

Table 4 shows the results of the study of the antipyretic effect of methanol extract of *M. oleifera* on hyperthermia induced by injection of a solution of brewer's yeast (20%) to rats. The oral administration of MEMO (250 and 500 mg/kg) significantly inhibited (P<0.01) hyperthermia induced by yeast. The antipyretic effect of MEMO (500 mg/kg) manifested from the 1st h and was remained significant up to 5th h of the post-treatment, while at the dose of 250 mg/kg the antipyretic effect appeared after 3rd h of the treatment and remained significant up to 5th h. A non-significant antipyretic effect at the dose 100 mg/kg was also observed. The percent pyrexia inhibition of all the tested groups is shown in **(Figure 1) (Table 4)**.

Figure 1: Percent of the antipyretic effect of MEMO in Brewer's yeast induced pyrexia test.



[A] paracetamol, 150mg/kg [B] MEMO 500mg/kg and [C] MEMO 250 mg/kg [D] MEMO 100 mg/kg. Values are reported as mean ± S.E.M (n=5). *P<0.05, **P<0.01, ***P<0.001 compared to control.

Table 4*:* Antipyretic effect of MEMO in Brewer's yeast induced pyrexia test.

Treatment Dose(mg/kg)	**Rectal temperature in rats after drug administration**						
	T0	**After 18 h**	**19h**	**20h**	**21h**	**22h**	**23h**
Normal saline 10(ml/kg)	36.56±0.23	38.88±0.18	38.74±0.17	38.56±0.17	38.32±0.12	38.50±0.14	38.56±0.15
MEMO100	36.27±0.11	38.95±0.17	38.66±0.18	38.67±0.18	38.27±0.11	38.46±0.13	38.60±0.15
MEMO 250	36.41±0.32	39.29±0.05	39.01±0.05	38.77±0.06	38.09±0.05**	38.19±0.09***	38.42±0.11***
MEMO 500	36.15±0.21	38.90±0.11	38.41±0.09***	38.29±0.08*	37.31±0.08***	37.77±0.06***	37.98±0.07***
Paracetamol 150	35.96±0.23	38.58±0.15	37.36±0.14***	37.3±0.15***	37.15±0.09***	36.70±0.22***	36.44±0.22***

Values are mean±SEM (n=5). *P<0.05, **P<0.01, ***P<0.001compared to control.

4. DISCUSSION

The safety studies on herbal medicines have been carried out by performing acute and sub-acute toxicity tests in laboratory animals [16]. Acute toxicity study showed that the methanol extract of *Moringa oleifera* Lam possessed high safety profile, as MEMO did not present noticeable signs of toxicity or mortality at the dose of 2000 mg/kg, in any animal during the

entire observation period. Hematological and biochemical parameters did not change significantly as compared to the control group. These results indicated that the MEMO is safe and explained the utilisation of the plant in traditional medicine.

Inflammation is a complex biological response of vascular tissues invasion by an infectious agent, antigen challenge, physical, chemical or traumatic damage. Although inflammation is a defence mechanism, the complex events and mediators involved in the inflammatory reactions can induce, maintain or aggravate many diseases [17]. Egg albumin induced rat paw edema is one of a commonly used primary test to evaluate the anti-inflammatory effect of natural products. Edema formation results from the synergistic action of inflammatory mediators such as histamine, serotonin and bradykinin produced under the effect of cycloxygenase-2 (COX-2) at the site of a local inflammatory insult leading to increased vascular permeability and blood flow [18,19]. Moreover, edema formation due to egg albumin in the rat paw is a biphasic event; the early phase of edema, which begins immediately after the administration of mine and serotonin. While the latter phase, occurring from 3 to 5 h after the administration of the irritant is induced by bradykinin, protease, prostaglandins and lysosome [20,21]. The anti-oedematous effect showed that MEMO significantly inhibited the formation of the paw edema during the first phase and significantly maintained in the second one. It can be suggested that this anti-inflammatory effect probably might be attributed to the inhibition of the release of pro-inflammatory mediators of acute inflammation, especially the prostaglandins by the methanol extract of leaves of *M. oleifera*.

Fever is a surrogate marker for disease activity in many infectious and inflammatory disorders. According to the classical view, the genesis of fever is induced by inflammatory mediators (i.e., cytokines, namely interleukin-1, interleukin-6, tumor necrosis factor, and others) that are predominantly released by activated peripheral mononuclear phagocytes and other immune cells [22,23]. Cytokines which are transported by the bloodstream could act at sites lacking a tight blood–brain barrier, the so-called circumventricular organs [24]. Alternatively, circulating cytokines could interact with their specific receptors on brain endothelial cells [25] or perivascular cells [26] and thereby stimulate these cells to release pyrogenic mediators into the abluminal brain tissue. It has been proposed that fever-promoting cytokines are transported from the blood into the brain via specific carriers [27]. An assumed manifestation of a febrile response produced by these mechanisms is termed as the humoral hypothesis of fever induction. Within the brain, prostaglandin E_2 (PGE_2), produced by cyclooxygenase (COX)-2, is regarded as the principle downstream mediator of fever [28] acting on thermosensitive or thermointegrative hypothalamic neurons.

The most likely cell type in the central nervous system responsible for producing PGE_2 is the microvascular endothelial cell, which expresses COX-2 exuberantly after stress. An effective febrifuge might interrupt pyrexogenesis at any step that connects peripheral inflammation with the central production of PGE_2. Stated differently, an antipyretic might blunt peripheral inflammation or depress central pyrogenic signals, or it may affect both. Inhibiting central production of PGE_2 is a well-known mechanism of antipyretic agents, but activated leukocytes and endothelial cells in peripheral areas of inflammation also represent potential drug targets.

The subcutaneous injection of brewer's yeast evoked pyrexia by ultimately increasing synthesis of prostaglandin and is considered as a valuable *in vivo* screening test for the assessment of antipyretic potential[29-31].The inhibition of prostaglandin synthesis could be the possible mechanism of antipyretic action as that of paracetamol and the inhibition of prostaglandin can be achieved by blocking the cyclooxygenase enzyme activity [32,33]. Further, it does not inhibit neutrophil activation. In supra-pharmacologic doses it inhibits NF-kB stimulation of inducible nitric oxide synthase [34]. The antipyretic effect of MEMO may be due to inhibiting

the enzyme cyclooxygenase and reducing the level of prostaglandin within the hypothalamus in pyrexia rats.

Phytochemical study of the plant *Moringa oleifera* showed the presence of phytochemical constituents like alkaloids, flavonoids, saponins, tannins and terpenoids. Furthermore, the presence of Sterols, carbohydrates, glycosides, Amino acid and proteins [35].Flavonoids have been demonstrated that they are able to inhibit a series of enzymes, which are activated in the course of the inflammatory process [36].Also, flavonoid such as vicenin-2, Tiliroside, Schaftoside, have been recognized as potent inhibitors of pro-inflammatory mediators in different studies [37-40]. Anti-inflammatory and antipyretic action of MEMO may be due to the presence of above phytoconstituents. Hence, the effect may be due to the synergistic effect or than single constituent [41,42].

5. CONCLUSION

Based on the results of this study, it could be confirmed that the methanol extract of leaves of *M.oleifera* contained secondary metabolites that showed outstanding anti-inflammatory and antipyretic activity, the results also provide scientific evidence for the use of this herbal in phytotherapy. The plant therefore could be regarded as a natural source of anti-inflammatory and antipyretic compounds and could be used as an alternative remedy for treatment of inflammatory related disorders and disease. Further studies are necessary to elucidate the mechanisms involved in its biological activity and isolate the chemical constituents responsible for these activities.

Conflict Of Interest

The authors confirm that this article content has no conflict of interest.

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